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2017 Rec'd PCT/PTO 23 APR 2001

FORM PTD-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER: 98 BA INS SAM U.S. APPL. NO. (If known, see 37 CFR 1.5) <div style="font-size: 1.5em; font-weight: bold;">09/830188</div>
INTERNATIONAL APPLICATION NO.: PCT/EP99/08031	INTERNATIONAL FILING DATE: 22 October 1999	PRIORITY DATE CLAIMED: 23 October 1998
TITLE OF INVENTION: CHELATING AGENTS FOR RADIOIMMUNOTHERAPY		
APPLICANT(S) FOR DO/EO/US: Jean-François GESTIN, Anthony LOUSSOUARN and Alain FAIVRE-CHAUVET		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1. <input checked="" type="checkbox"/> 2. <input type="checkbox"/> 3. <input checked="" type="checkbox"/> 4. <input checked="" type="checkbox"/> 5. <input checked="" type="checkbox"/> 6. <input type="checkbox"/> 7. <input type="checkbox"/> 8. <input type="checkbox"/> 9. <input type="checkbox"/> 10. <input type="checkbox"/> 11. <input checked="" type="checkbox"/> 12. <input type="checkbox"/> 13. <input type="checkbox"/> 14. <input type="checkbox"/> 15. <input type="checkbox"/> 16. <input checked="" type="checkbox"/>	This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. A copy of the International Application as filed (35 U.S.C. 371(c)(2)) a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. (see attached copy of PCT/IB/308) c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). A translation of the International Application into English (35 U.S.C. 371(c)(2)). Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). Item 11. to 16. below concern document(s) or information included: An Information Disclosure Statement under 37 CFR 1.97 and 1.98. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. A FIRST preliminary amendment. A SECOND or SUBSEQUENT preliminary amendment. A substitute specification. A change of power of attorney and/or address letter. Other items or information:	
International Preliminary Examination Report (PCT/IPEA/409) International Search Report (PCT/ISA/210) Abstract of the Disclosure on a separate sheet Application Data Sheet		



Y&T 9/2000

PC7



PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jean-Francois GESTIN et al.

Serial No. 09/830,188

GROUP Unassigned

Filed April 23, 2001

Examiner Unassigned

CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

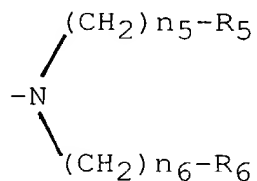
Sir:

The following amendments to the claims are submitted prior to examination of the above-referenced application.

IN THE CLAIMS:

Please amend the claims as follows:

3. (amended) Compounds according to claim 1, characterized in that at least one, and more preferably two of R₁, R₂, R₃ and R₄, represent a group

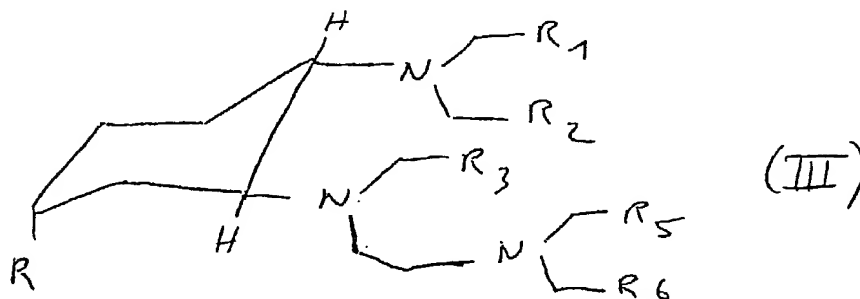


GESTIN ET AL. S.N. 09/830,188

4. (amended) Compounds according to claim 1, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in claim 1, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding.

5. (amended) Compounds according to claim 1, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides.

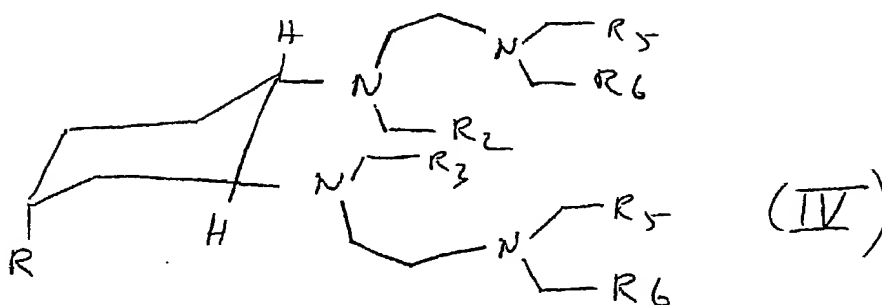
6. (amended) Compounds of the following formula (III) :



GESTIN ET AL. S.N. 09/830,188

in which R_1 , R_2 , R_3 , R_5 and R_6 independently from each other represent $-\text{COOH}$ or $-\text{PO}(\text{OH})_2$, and R is a group as defined in claim 2.

8. (amended) Compounds of the following formula (IV) :



wherein R_2 , R_5 and R_6 , independently from each other, represent $-\text{COOH}$ or $-\text{PO}(\text{OH})_2$, and R is a group as defined in claim 2.

10. (amended) Complexes between a compound according to claim 1, and a radioactive element.

13. (amended) Use of a complex according to claim 11, for the manufacture of a medicament for radioimmunotherapy, such as for the treatment of cancers,

GESTIN ET AL. S.N. 09/830,188

and more particularly for the treatment against metastase proliferation.

14. (amended) Pharmaceutical compositions characterized in that they comprise an effective amount of a complex according to claim 1, in association with a suitable pharmaceutical carrier.

16. (amended) Complexes according to claim 15, characterized in that the compound is chosen among those compounds wherein the group R comprises:

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

GESTIN ET AL. S.N. 09/830,188

17. (amended) Use of a complex according to claim 15, for carrying out diagnosis methods such as radioimmunosciintigraphy.

18. (amended) Use of a compound of formula (I) as defined in claim 1, included compounds CDTPA and CTTHA, for:

- the manufacture of a medicament useful as antalgic, or for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism,

- carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not,

- carrying out a process for the radionuclides purification, said compound being bound to a solid phase,

- carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

Respectfully submitted,

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May 31, 2001

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"MARKED-UP VERSION SHOWING CHANGES MADE"

$$\begin{array}{l}
 \text{H} \\
 | \\
 \text{N} \begin{cases} (\text{CH}_2)_{n_1} - \text{R}_1 \\ (\text{CH}_2)_{n_2} - \text{R}_2 \end{cases} \\
 | \\
 \text{N} \begin{cases} (\text{CH}_2)_{n_3} - \text{R}_3 \\ (\text{CH}_2)_{n_4} - \text{R}_4 \end{cases} \\
 | \\
 \text{H}
 \end{array}
 \quad (\text{I})$$

- n_1, n_2, n_3 and n_4 , independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,

$$\begin{array}{l} \cdot -\text{COOH}, \\ \cdot -\text{PO}(\text{OH})_2, \\ \text{-N} \begin{array}{l} \diagup (\text{CH}_2)_n\text{-R}_5 \\ \diagdown \text{Y} \end{array} \end{array}$$

wherein n_5 represents an integer from 1 to 5, preferably from 1 to 3, R_5 represents $-\text{COOH}$ or $-\text{PO}(\text{OH})_2$, and Y represents H or a group $-(\text{CH}_2)_{n_6}-\text{R}_6$ in which n_6 represents an integer from 1 to 5, preferably from 1 to 3, and R_6 represents $-\text{COOH}$ or $-\text{PO}(\text{OH})_2$,

$$\begin{array}{c} \text{---N} \begin{array}{l} \diagup (\text{CH}_2)_{n_5}\text{---R}_5 \\ \diagdown \text{Y} \end{array} \end{array}$$

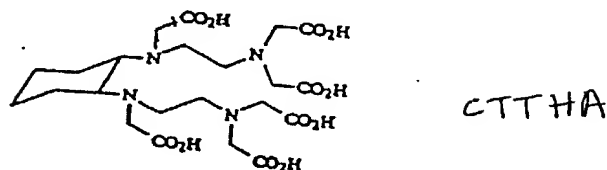
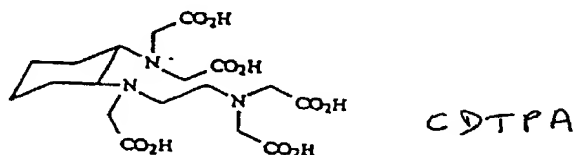
- R represents :

. H, or -NHCOCH₃, or

. a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

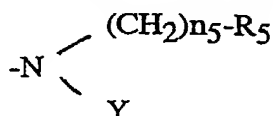
: a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



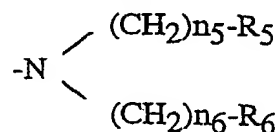
2. Compounds according to claim 1, characterized in that :

- when R₁, R₂, R₃ or R₄ represents -COOH or -PO(OH)₂, then n₁, n₂, n₃ or n₄ represents 1 respectively,
- when R₁, R₂, R₃ or R₄ represents a group



- then n₁, n₂, n₃ or n₄ represents 2 or 3 respectively, and preferably 2,
- n₅, and optionally n₆, represents 1.

3. Compounds according to ^{a1}[claims 1 or 2], characterized in that at least one, and more preferably two of R₁, R₂, R₃ and R₄, represent a group



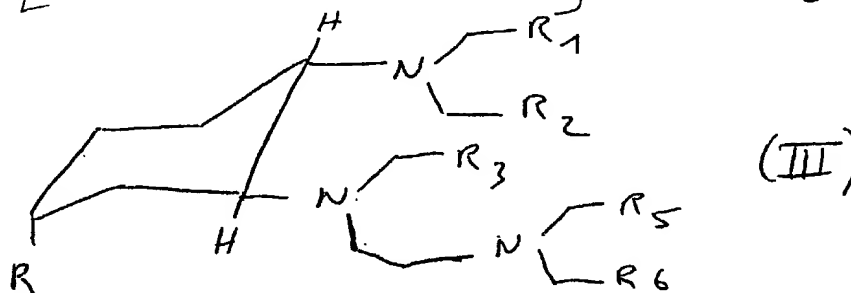
[wherein n₅, n₆, R₅ and R₆ are defined in claims 1 or 2].

4. Compounds according to ^{a1}[anyone of claims 1 to 3], characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in

claim 1, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding.

5 5. Compounds according to ^{cl 1} [anyone of claims 1 to 3], characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in claim 1.

10 6. Compounds [according to anyone of claims 1 to 5] of the following formula (III) :

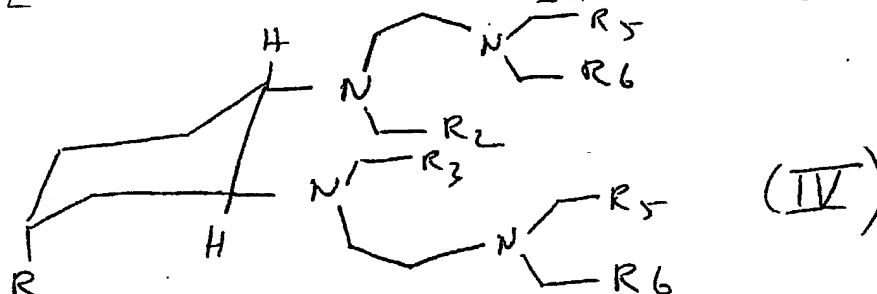


20 in which R₁, R₂, R₃, R₅ and R₆ independently from each other represent -COOH or -PO(OH)₂, and R is a group as defined in ^{cl 2} [claims 2 to 5].

25 7. Compounds according to claim 6, of formula (III) wherein :

- . R₁ = R₅ = R₆ = COOH and R₂, = R₃ = PO(HO)₂, or
- . R₁ = R₂ = R₃ = R₅ = R₆ = COOH, or
- . R₁ = R₂ = R₃ = R₅ = R₆ = PO(OH)₂.

30 8. Compounds [according to anyone of claims 1 to 5], of the following formula (IV) :



35 wherein R₂, R₅ and R₆, independently from each other, represent -COOH or -PO(OH)₂, and R is a group as defined in ^{cl 2} [claims 2 to 5].

. $R_2 = R_3 = \text{PO}(\text{OH})_2$, and $R_5 = R_6 = \text{COOH}$, or

$$. R_2 = R_3 = R_5 = R_6 = \text{COOH, or}$$
$$. R_2 = R_3 = R_5 = R_6 = PO(OH)_2.$$

11. Complexes according to claim 10, characterized in that said radioelements are α or β emitter radiometals.

12. Complexes according to claim 11, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound, in a first step of the treatment, to epitopes on the surface of specific cells in the organism, ..

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

13. Use of a complex according to ^{cl 11}claims 11 or 12, for the manufacture of a medicament for radioimmunotherapy, such as for the treatment of cancers, and more particularly for the treatment against metastase proliferation.

14. Pharmaceutical compositions characterized in that they comprise an effective amount of a complex according to ¹⁶claims 11 or 17, in association with a suitable pharmaceutical carrier.

15. Complexes according to claim 10, characterized in that the radioelements are γ emitter radiometals.

16. Complexes according to claim 15, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

17. Use of a complex according to ^{cl 15} claims 15 or 16, for carrying out diagnosis methods such as radioimmunoscintigraphy.

18. Use of a compound of formula (I) as defined in claim 1 to 9, included compounds CDTPA and CTTHA, for :

- the manufacture of a medicament useful as antalgic, or for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism,

- carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not,

- carrying out a process for the radionuclides purification, said compound being bound to a solid phase,

- carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

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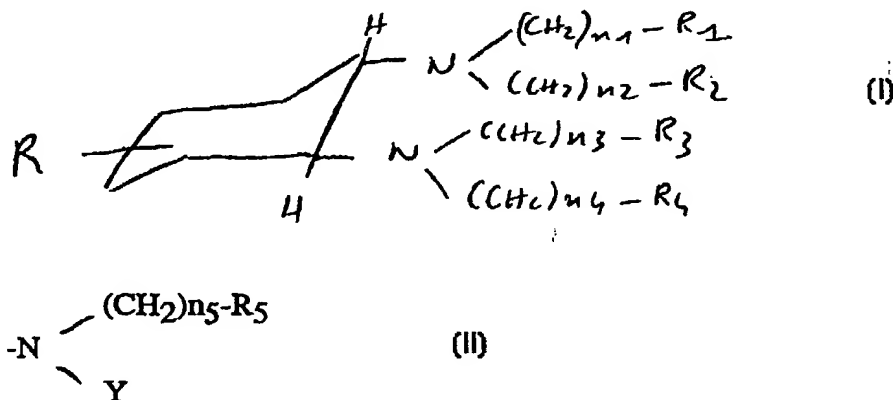
<p>(51) International Patent Classification ⁷ : C07F 9/38, C07C 229/16, A61K 51/10, 51/08</p>	<p>A1</p>	<p>(11) International Publication Number: WO 00/24751 (43) International Publication Date: 4 May 2000 (04.05.00)</p>
<p>(21) International Application Number: PCT/EP99/08031 (22) International Filing Date: 22 October 1999 (22.10.99) (30) Priority Data: 98402648.4 23 October 1998 (23.10.98) EP (71) Applicant (for all designated States except US): I.N.S.E.R.M. (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE) [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cedex 13 (FR). (72) Inventors; and (75) Inventors/Applicants (for US only): GESTIN, Jean-François [FR/FR]; 5, chemin de la Coulée, F-44470 Mauves-sur-Loire (FR). LOUSSOUARN, Anthony [FR/FR]; 53, rue Fauré, F-44000 Nantes (FR). FAIVRE-CHAUVEY, Alain [FR/FR]; 24, rue E. Zola, F-44300 Reze (FR). (74) Agents: DEMACHY, Charles et al.; Grosset-Fournier & Demachy, 20, rue de Maubeuge, F-75009 Paris (FR).</p>		<p>(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.</p>

(54) Title: CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

(57) Abstract

The invention relates to compounds of formula (I): in which: n_1, n_2, n_3 and n_4 , represent an integer from 1 to 5, R_1, R_2, R_3 and R_4 , independently from each other, represent $-\text{COOH}$, $-\text{PO}(\text{OH})_2$, at least one of R_1, R_2, R_3 or R_4 represents a group (II), wherein n_5 represents an integer from 1 to 5, R_5 represents $-\text{COOH}$ or $-\text{PO}(\text{OH})_2$, and Y represents H or a group $-(\text{CH}_2)_{n_6}-R_6$ in which n_6 represents an integer from 1 to 5, and R_6 represents $-\text{COOH}$ or $-\text{PO}(\text{OH})_2$, R represents H, or

a group carrying a function linked to molecules able to bind with epitopes at the surface of cells. The invention also relates to the processes of preparation of said compounds, and to their use in pharmaceutical compositions and in diagnosis methods.



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CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

5 The invention relates to compounds useful as chelating agents, complexes between said compounds and radioelements, and to their uses, in particular in pharmaceutical compositions and compositions for the diagnosis of pathologies such as cancers.

10 Immunotherapy with radiolabeled antibodies should allow fairly specific targeting of certain cancers (Schubiger et al., 1996; Parker, 1990). However, iodine-131 (Bardies et al., 1992; Stein et al., 1995) may not be the best isotope for tumor therapy because of its limited specific activity, low beta-energy, relatively long half-life and strong gamma emission.

15 Another approach to improving therapeutic efficacy is the use of replacement isotopes with better physical properties. Chelators that can hold radiometals with high stability under physiological conditions are essential to avoid excessive radiation damage to non-target cells. Moreover, the development of new bifunctional chelating agents is essential for this purpose. Thus synthesis of new chelating agents able to bind radiometals such as
20 rhenium-188, yttrium-90, samarium-153 or Bismuth-213 and in general all the α and β particles emitters will be required to possess sufficiently stable chelators.

Accordingly, one of the aim of the invention is to provide chelating agents forming stable complexes *in vivo* with the numerous potential candidates for such applications.

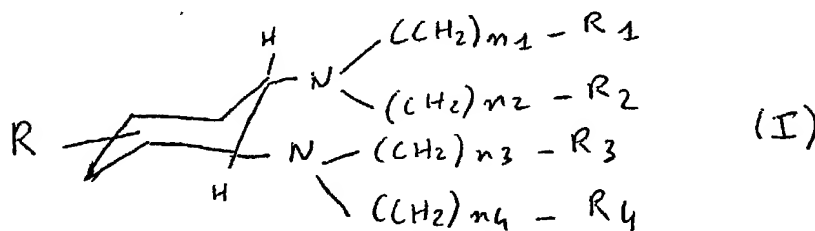
25 The stability of a non-macrocyclic ligand can be favourably influenced by the preorganization of the open chain. In fact, a semi-rigid structure such as that of *trans* 1-2 diaminocyclohexane limits the rotation of the ethylene bridge, so that the purpose of the cyclohexane design is to preorient the four pendent arms in a skew position.

30 A first investigation (Mease et al., 1990), which was guided by a study performed on polyaminocarboxylic acid ligands incorporating the skeleton of ethylenediaminetetraacetic acid (EDTA) in a cyclohexane structure, showed the influence of this semi-rigid structure on the stability of the resulting complexes. A second study (Goeckeler et al., 1987) of the stability of lanthanides as ^{153}Sm -polyaminophosphonic acid complexes showed that ethylenediamine
35 tetramethylphosphonic acid (EDTMP) derivatives allow stable quantitative ^{153}Sm chelation.

The (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride compound, the structural derivative of *trans*-1,2-diaminocyclohexane, have been prepared (Gestin et al., 1997; Loussouarn, et al., 1998). This intermediate, which is functionalized at position 4 of the cycle by a protected amine termination (Meares et al., 1984) for future covalent attachment to biomolecules, allows the introduction of different chelating groups via the free amines.

The Inventors have developed a new simple and efficient synthesis pathway from *trans*-1,2-diaminocyclohexane to provide access to a new class of semi-rigid chelating agents. This same reactional scheme applies to the reactional intermediary, (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride, which allows the synthesis of these same chelating agents, though functionalized back of the cycle by a termination allowed coupling to an antibody or any other biological substance such as a hapten.

The present invention relates to compounds of the following formula (I) :



in which :

- n_1 , n_2 , n_3 and n_4 , independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,

- R_1 , R_2 , R_3 and R_4 , independently from each other, represent :

. -COOH,

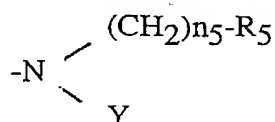
. -PO(OH)₂,

. (CH₂)_{n5}-R₅

-N
Y

wherein n_5 represents an integer from 1 to 5, preferably from 1 to 3, R_5 represents -COOH or -PO(OH)₂, and Y represents H or a group -(CH₂)_{n6}-R₆ in which n_6 represents an integer from 1 to 5, preferably from 1 to 3, and R_6 represents -COOH or -PO(OH)₂,

provided that at least one of R_1 , R_2 , R_3 or R_4 represents a group



such as defined above,

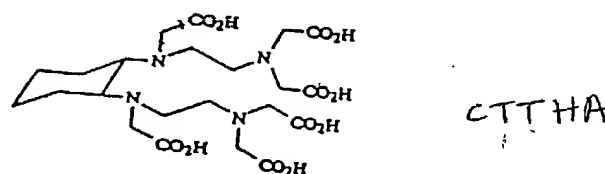
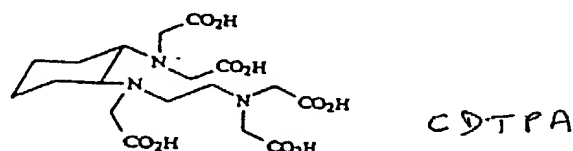
- R represents :

. H, or -NHCOCH_3 , or

. a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

. a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

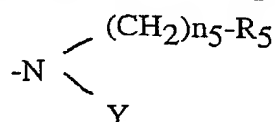
the two following compounds, CDTPA and CTTHA, being excluded :



The invention relates more particularly to compounds of formula (I) such as defined above, characterized in that :

- when R_1 , R_2 , R_3 or R_4 represents -COOH or -PO(OH)_2 , then n_1 , n_2 , n_3 or n_4 represents 1 respectively,

- when R_1 , R_2 , R_3 or R_4 represents a group



then n_1 , n_2 , n_3 or n_4 represents 2 or 3 respectively, and preferably 2,

- n_5 , and optionally n_6 , represents 1.

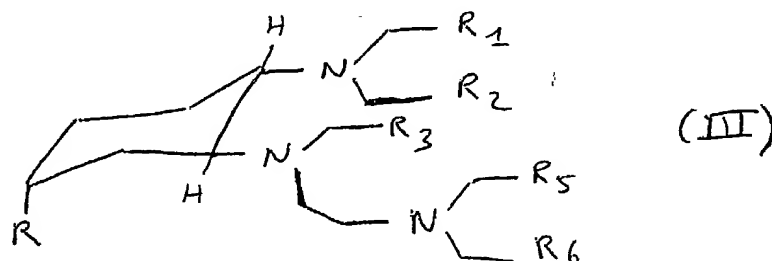
- . alcohol group, such as -OH,
- . amino group, such as -NH₂, -NO₂,
- . aldehyde group, such as -CHO,
- . carboxylic group, such as -COOH,
- . anhydride group, such as -CO-O-CO-R",
- . -CO-CH₂X, wherein X represents an halogen atom, such as Cl or Br,
- . -CO-X, wherein X represents an halogen atom, such as Cl or Br,
- . a diazonium ion N₂⁺,
- . an activated ester, such as -COOR", R" = ethyl or N-hydrosuccinimide,
- . a sulfonic group, such as SO₃H,
- . a thiocyanate group, such as -NCS, or an isocyanate -NCO, or a -NH-NCS group
- . a thiol group, such as -SH,
- . a disulfure group, such as -S-S-R".

The invention also concerns compounds of formula (I) or (II) such as defined above, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined above, and more particularly R represents a group chosen among the following groups :

- . -O-CO-R',
- . -NH-CO-R',
- . -NH-CS-R',
- . -CH=N-R',
- . -CO-NH-R',
- . -CO-CH₂-NH-R',
- . -N=N-NH-R',
- . -SO₂-NH-R',
- . -NH-CS-NH-R',
- . -thioether-R',
- . -CO-S-R',
- . -CO-CH₂-S-R',
- . -S-S-R',
- . -NH-CH₂-R',
- . -CO-NH-N=CH-R',
- . -CS-NH-N=CH-R',

wherein R' represents said molecule.

The invention concerns more specifically compounds such as described above of the following formula (III) :

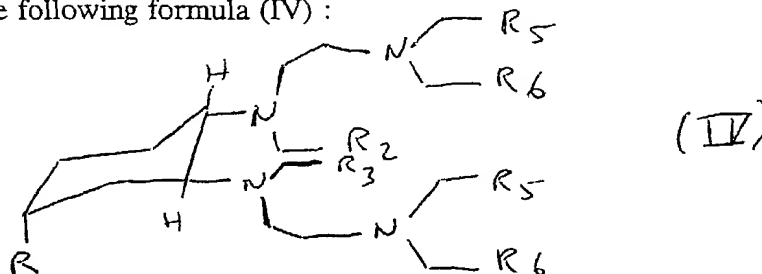


in which R₁, R₂, R₃, R₅ and R₆ independently from each other represent -COOH or -PO(OH)₂, and R is a group as defined above.

Preferred compounds of formula (III) are such that :

- . R₁ = R₅ = R₆ = COOH and R₂, = R₃ = PO(HO)₂, or
- . R₁ = R₂ = R₃ = R₅ = R₆ = COOH, or
- . R₁ = R₂ = R₃ = R₅ = R₆ = PO(OH)₂.

The invention also concerns more specifically compounds such as described above, of the following formula (IV) :



wherein R₂, R₅ and R₆, independently form each other, represent -COOH or -PO(OH)₂, and R is a group as defined above.

Preferred compounds of formula (IV) are such that :

- $R_2 = R_3 = \text{PO}(\text{OH})_2$, and $R_5 = R_6 = \text{COOH}$, or
- $R_2 = R_3 = R_5 = R_6 = \text{COOH}$, or
- $R_2 = R_3 = R_5 = R_6 = \text{PO}(\text{OH})_2$.

The invention also relates to complexes between a compound such as described above, and a radioactive element, said complexes resulting from the association of said radioelement with the -COOH and/or -PO(OH)₂ groups of said compound (the bonds between said radioelement and said compound being ionic bonds).

The above-mentioned radioelements are more particularly α , β or γ emitter radiometals, and preferably from the groups of actinides or lanthanides.

The invention relates more particularly to complexes such as described above, characterized in that said radioelements are α or β emitter radiometals (susceptible to be used in therapy, and more particularly in radioimmunotherapy in the frame of cancer treatments).

Advantageously, α emitter radiometals are chosen among the followings : Actinium 225, Bismuth 213.

Advantageously, β emitter radiometals are chosen among the followings : ^{33}P , ^{199}Au , ^{121}Sn , ^{177}Lu , ^{67}Cu , ^{105}Rh , ^{47}Sc , ^{77}As , ^{153}Sm , ^{159}Gd , ^{143}Pr , ^{186}Re , ^{111}Ag , ^{149}Pm , ^{109}Pd , ^{166}Ho , ^{32}P , ^{188}Re , ^{194}Ir , ^{142}Pr , ^{90}Y .

Preferred complexes with radiometals used in therapy, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the treatment) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

The invention also concerns the use of a complex such as described above, for the manufacture of a medicament for radioimmunotherapy (also called radiopharmaceutical), in particular for the treatment of cancers, or for the treatment against metastase proliferation.

More particularly, the invention relates to the use of a complex such as defined above, for the manufacture of a medicament for the treatment of :

- lung cancer, said complex preferably being such that it comprises a radioelement chosen among : ^{188}Re , ^{186}Re , ^{153}Sm , ^{67}Cu and ^{90}Y , and wherein R comprises an antibody specific for lung cancer cells, such as Anti N-CAM Antibody, Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti N-CAM-679 Bispecific antibody, Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti N-CAM-734 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- liver and pancreatic cancers, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for liver and pancreatic cancer cells, such as antibodies and haptens described above in the case of lung cancer,

- ovarian cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for ovarian cancer cells, such as OC125, MOV18, MOV19, OVTL3, or an hapten chosen among OC125-679 Bispecific antibody, MOV18-679 Bispecific antibody, MOV19-679 Bispecific antibody, OVTL3-679 Bispecific antibody, OC125-734 Bispecific antibody, MOV18-734 Bispecific antibody, MOV19-734 Bispecific antibody, OVTL3-734 Bispecific antibody,

- bladder cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and

wherein R comprises an antibody specific for bladder cancer cells, such as AC48-127, or an hapten chosen among 48-127 Bispecific antibody, 48-127-679 Bispecific antibody, 48-127-734 Bispecific antibody,

- colorectal cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for colorectal cancer cells, such as Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- thyroid medullary cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for thyroid medullary cancer cells, such as Anti CEA Antibody, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti CEA-734 Bispecific antibody,

- lymphoma, said complex preferably being such that it comprises a radioelement chosen among : ^{213}Bi , ^{225}Ac , ^{153}Sm , and ^{67}Cu , and wherein R comprises an antibody specific for lymphoma cells, such as specific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19, CD37, or an hapten such as bispecific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19-679, CD37-679, CD19-734, CD37-734,

- myeloma, said complex preferably being such that it comprises a radioelement chosen among : ^{213}Bi , ^{225}Ac , ^{153}Sm , and ^{67}Cu , and wherein R comprises an antibody specific for myeloma cells, such as specific antibody against expressed antigens surfaces myeloma cells, e.g. BB4, or an hapten such as bispecific antibody against expressed antigens surfaces myeloma cells, BB4-679, BB4-734,

- osteoarticular pathology, particularly in bone cancer extension balance.

The invention also concerns pharmaceutical compositions characterized in that they comprise an effective amount of a complex such as described above, in association with a suitable pharmaceutical carrier.

Pharmaceutical compositions according to the invention are more particularly characterized in that they are in a form suitable for an IV or IP administration in located areas.

Preferred pharmaceutical compositions according to the invention, are characterized in that the daily dosage is comprised between 1 and 100MBq /kg, e.g. between 3,7 and 74MBq/kg.

The invention also relates to complexes, as defined above, between a compound such as described above, and a radioactive element, characterized in that the radioelements are γ emitter radiometals (i.e. radiometals susceptible to be used in diagnosis methods, such as radioimmunoscinigraphy).

Advantageously, said radiometals are chosen among ^{111}In , $^{99\text{m}}\text{Tc}$, ^{64}Cu .

Preferred complexes with radiometals used in diagnosis, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,
- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,
- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

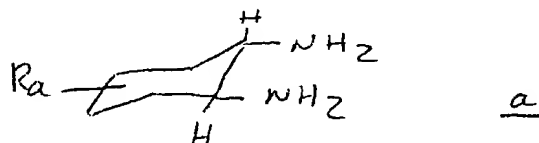
More particularly, the invention concerns the use of a complex such as defined above, for carrying out the following diagnosis methods by radioimmunosciintigraphy :

- diagnosis of cancers, such as cited above, the complex used being preferably such that it comprises ^{111}In , or $^{99\text{m}}\text{Tc}$ as radioelements, and R comprises an antibody or a hapten specific for such cancer cells, such as antibodies or haptens mentioned above in the frame of the cancers listed above,
- diagnosis of cardiovascular diseases, such as graft rejection, myocardic infarcts,
- diagnosis of cerebral diseases,
- diagnosis of renal diseases, in particular in the study of individual kidney functions, location of ectopic kidney, renal filtration and secretion troubles,
- vascular diseases, such as embolism and thrombosis, the complex used being preferably such that it comprises ^{111}In , or $^{99\text{m}}\text{Tc}$ as radioelements, and R comprises an antibody such as anti platelets or anti fibrin antibodies.

A process for the preparation of compounds according to the invention, comprises the following steps :

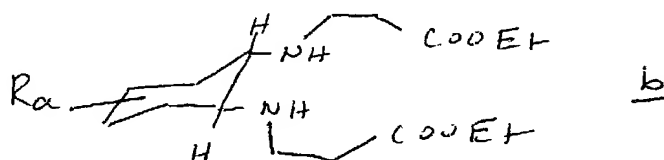
The invention also relates to processes for preparing compounds and complexes as described above. A process for the preparation of compounds according to the invention, comprises the following steps :

- contacting trans-1,2-diaminocyclohexane of the following formula a :

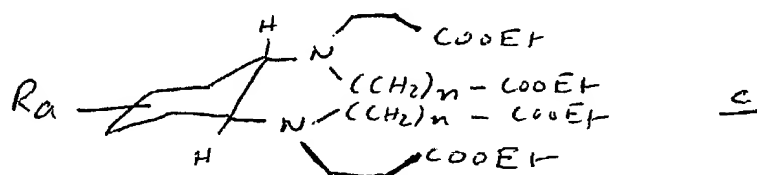


wherein R_a is H or NHCOCH_3 ,

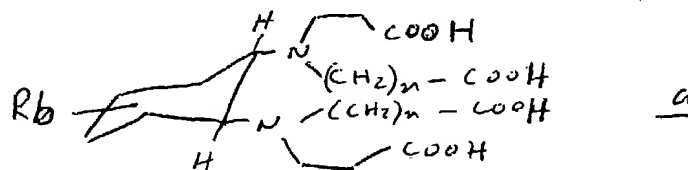
* either with vinyl propionate, preferably by stirring 20h at room temperature, leading to the following compound b



. contacting compound b with $\text{X}-(\text{CH}_2)_n-\text{COOEt}$, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound c

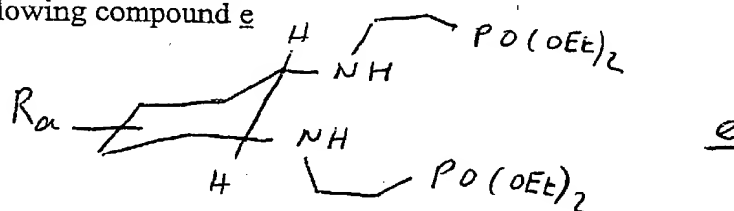


. treating compound c with HCl, preferably 6N HCl at reflux overnight, leading to the following compound d

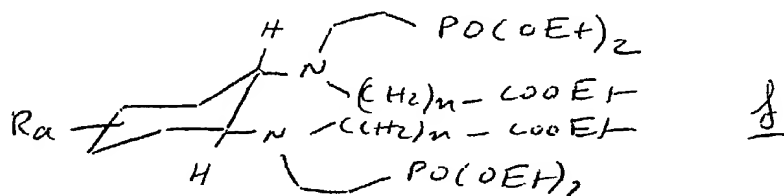


wherein R_b represents H or NH_2 ,

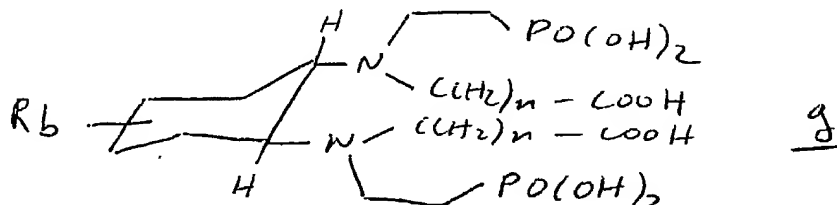
* or with diethyl vinyl phosphonate, preferably by stirring 15h at reflux, leading to the following compound e



. contacting compound e with $X-(CH_2)_n-COOEt$, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound f

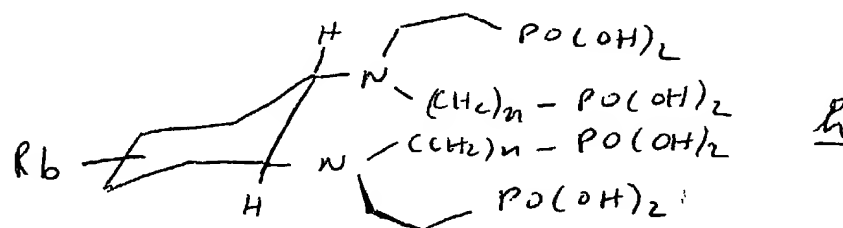


. treating compound f with HCl, preferably 6N HCl at reflux overnight, leading to the following compound g



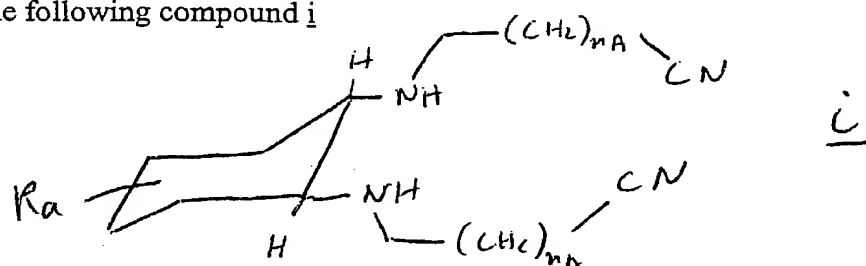
wherein R_b represents H or NH_2 ,

. if desired, treating compound g with phosphorous acid, preferably by stirring 30 mn at $80^\circ C$, leading to the following compound h

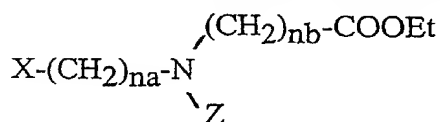


Another process for the preparation of compounds according to the invention, comprises the following steps :

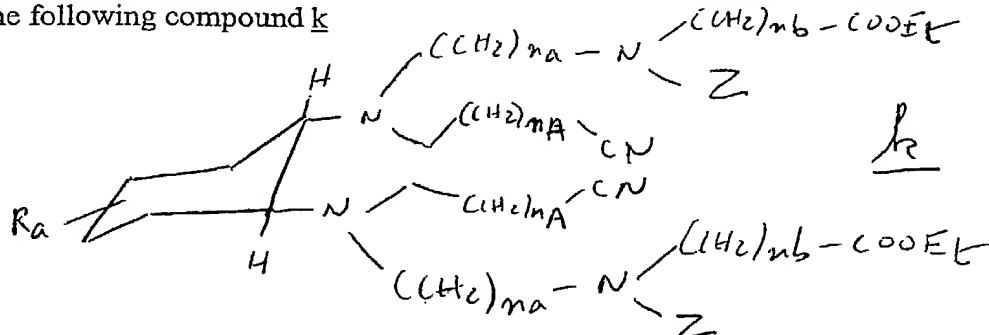
- contacting the compound of formula a described above with a compound of formula $H_2C=CH-(CH_2)_{nA}-CN$ wherein $nA = 0$ (acrylonitrile), or nA represents a integer from 1 to 3, preferably at room temperature during 20h, leading to the following compound i



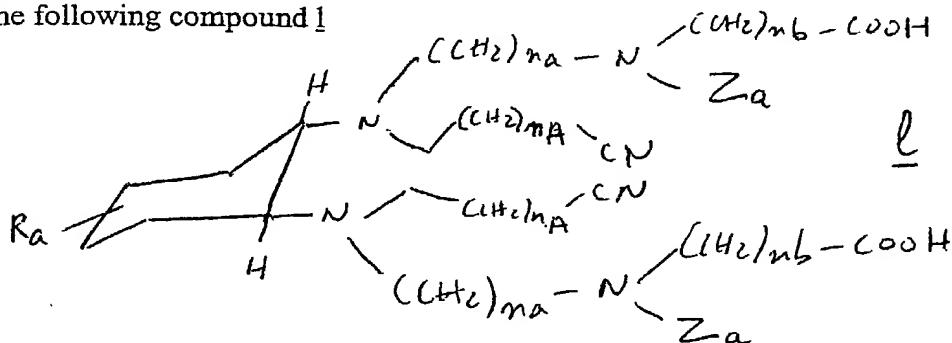
- contacting compound i with the following compound j



wherein X represents an halogen atom, na and nb, independently from each other represent an integer from 1 to 5, Z represents H or (CH₂)_{nc}-COOEt, and nc represents an integer from 1 to 5, preferably at 70°C during 2 days, leading to the following compound k



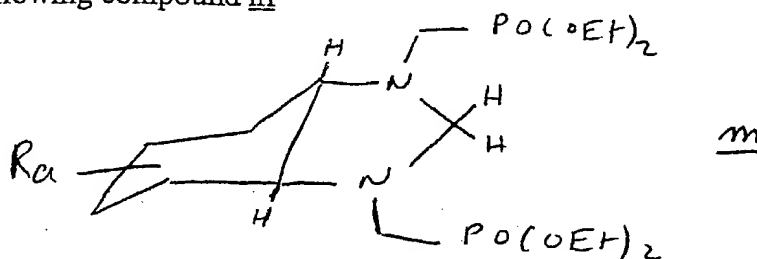
treating compound k with HCl, preferably 6N HCl at reflux overnight, leading to the following compound l



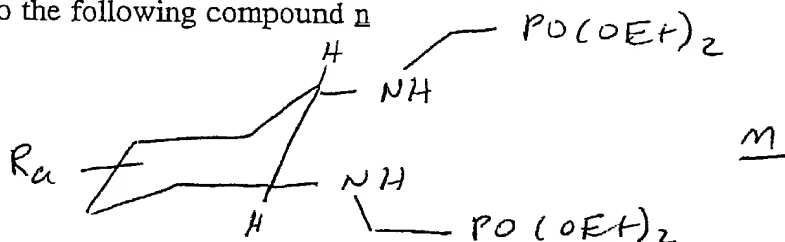
wherein Za represents H or -(CH₂)_{nb}-COOH, na, na and nb being such as defined above, and R_b represents H or NH₂.

Another process for the preparation of compounds according to the invention, comprises the following steps :

- contacting the compound of formula a described above with paraformaldehyde and diethylphosphite, preferably in THF at reflux during 4h, leading to the following compound m

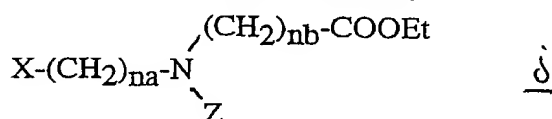


- treating compound m with HCl, preferably 3N HCl in MeOH at 50°C overnight, leading to the following compound n

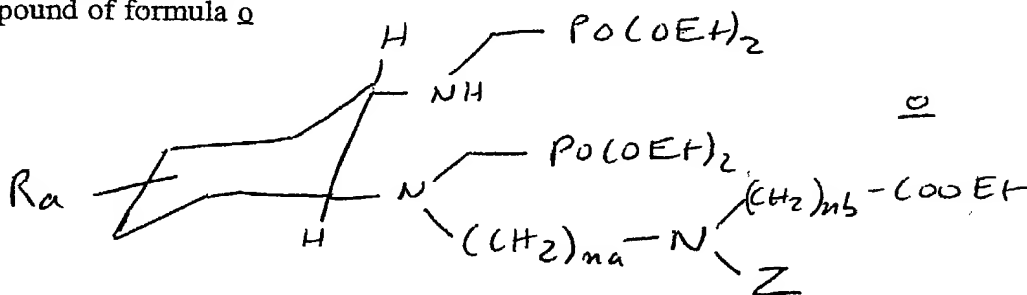


10 - contacting compound n :

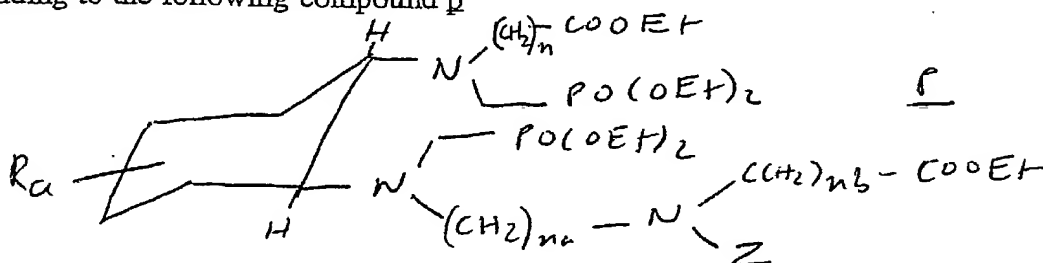
* either with 1 equivalent of compound j



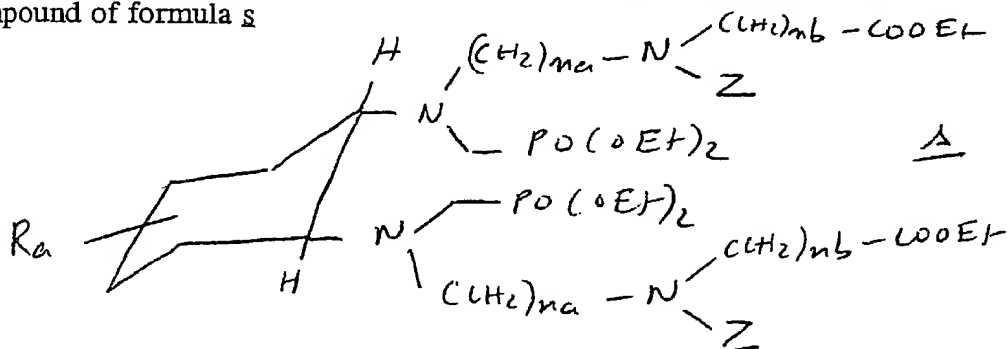
as described above, preferably at 70°C during 2 days, leading to the following compound of formula o



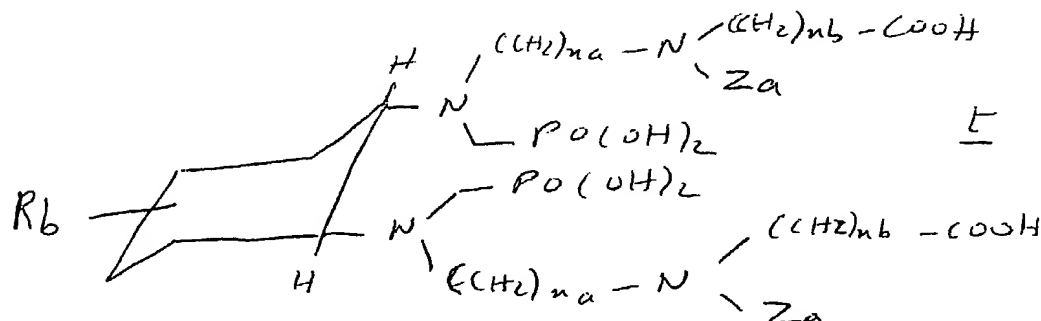
25 - contacting compound o with X-(CH2)n-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound p



35 - treating compound p with HCl, preferably 6N HCl at reflux overnight, leading to the following compound q

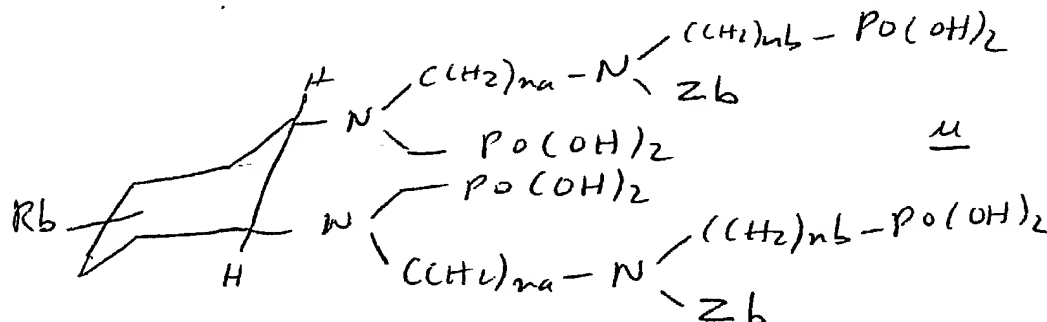


. treating compound s with HCl, preferably 6N HCl at reflux overnight, leading to the following compound t



wherein Z_a represents H or $-(CH_2)_{nb}-COOH$, na , nb and n being such as defined above, R_b represents H or NH_2 ,

. if desired, treating compound t with phosphorous acid, preferably by stirring 30 mn at $80^\circ C$, leading to the following compound u



wherein Z_b represents H or $-(CH_2)_{nb}-PO(OH)_2$.

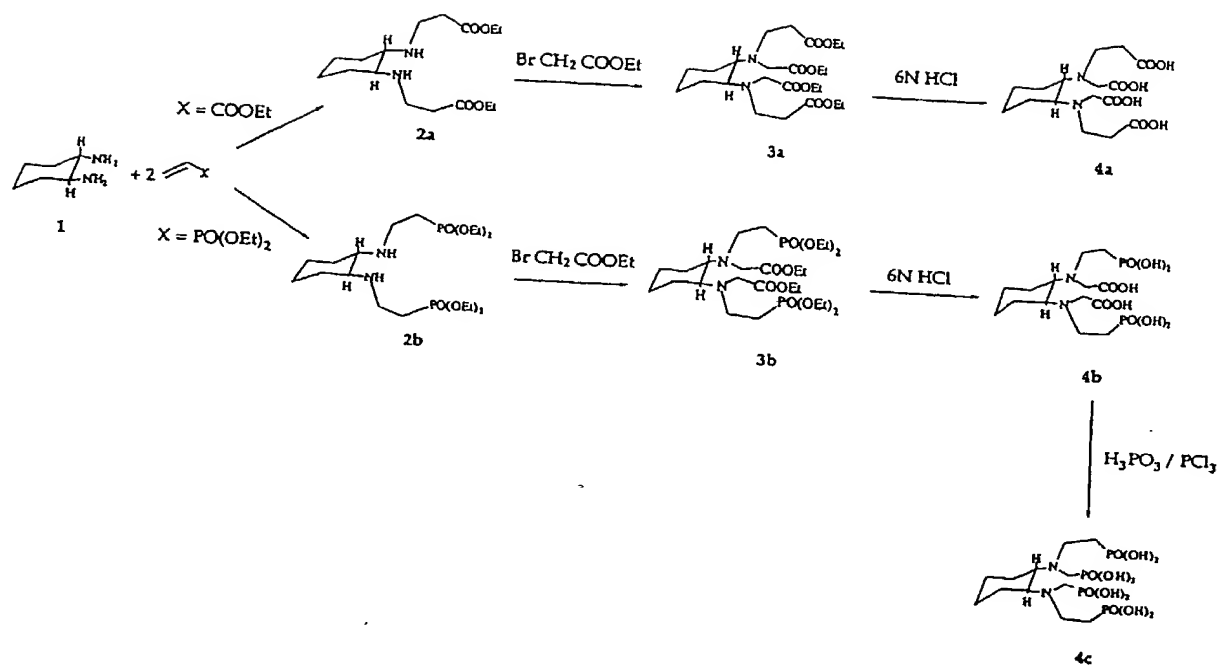
Compound of formula a can be obtained according to the method described in Gestin et al., 1997, and Loussouarn et al., 1998.

Compounds wherein R_b represents NH_2 obtained according to the processes described above, can then be transformed in order to correspond to compounds of formula (I) wherein R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules as defined above.

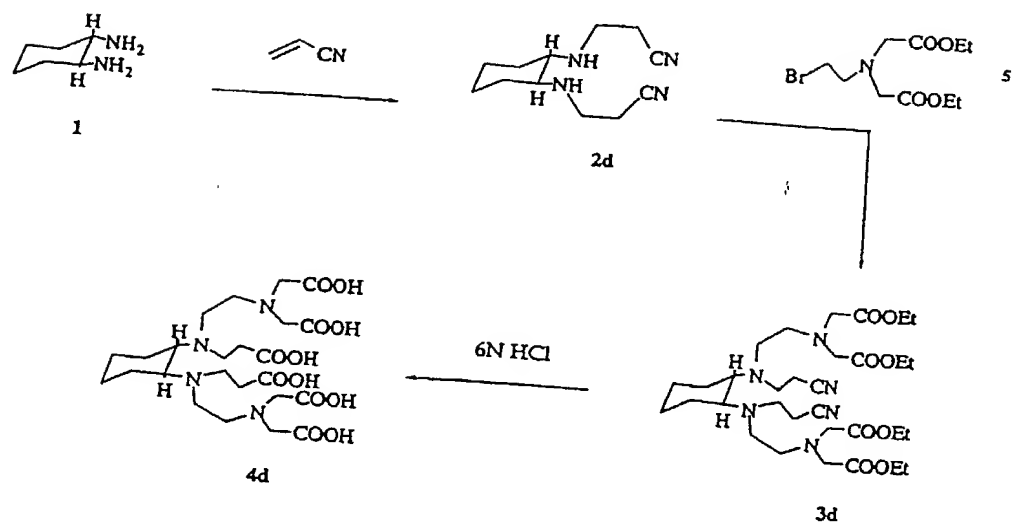
By way of example, compounds of formula (I) wherein R represents $-N=C=S$, can be obtained by treatment of said compounds wherein R_b represents NH_2 with $CSCl_2$, preferably in acidic or basic conditions.

Compounds of formula (I) wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

- The first depicted in schemes I and II was the Michael type addition of primary amines to some vinylic derivatives to provide monoaddition with high selectivity (Bergeron et al., 1981), allowing N-alkylation to be envisaged at this step. In strategy depicted in scheme I, compounds **2a** and **2b** were alkylated by ethyl bromoacetate under conditions recommended by Studer (Studer and Meares, 1992) (KI and Na₂CO₃) to give tetraesters **3a** and **3b**. Acid-catalysed hydrolysis of the ester functions was performed in 3M hydrochloric acid to give the tetracarboxylic acid **4a** and the mixed acid **4b**. At last, in order to generate the structure **4c**, carboxylic functions were converted into phosphonic functions using H₃PO₃/PCl₃ according to the method of Krüger and Bauer (Krüger and Bauer, 1972). The other strategy described in scheme II required preparation of protected bis-carboxymethylated amino ethyl bromide. In view to convenience of deprotecting ethyl esters by acid-catalysed hydrolysis, *N,N*-bis(ethylacetate)-2-bromoethyl-amine was prepared according to the Williams and Rapoport's procedure (Williams and Rapoport, 1994) with minor modifications. N-alkylation of **2d** with the branching group in a mixed solvent system (CH₃CN/EtOH) at 70°C gave **3d** in 60% yield. Acid-catalysed hydrolysis of the ester functions as well as nitriles is the more convenient method (Ornstein et al., 1989), of hexacarboxylic acid **4d** preparation.

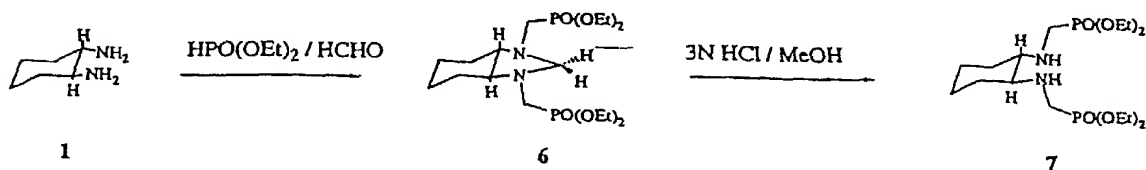


scheme I



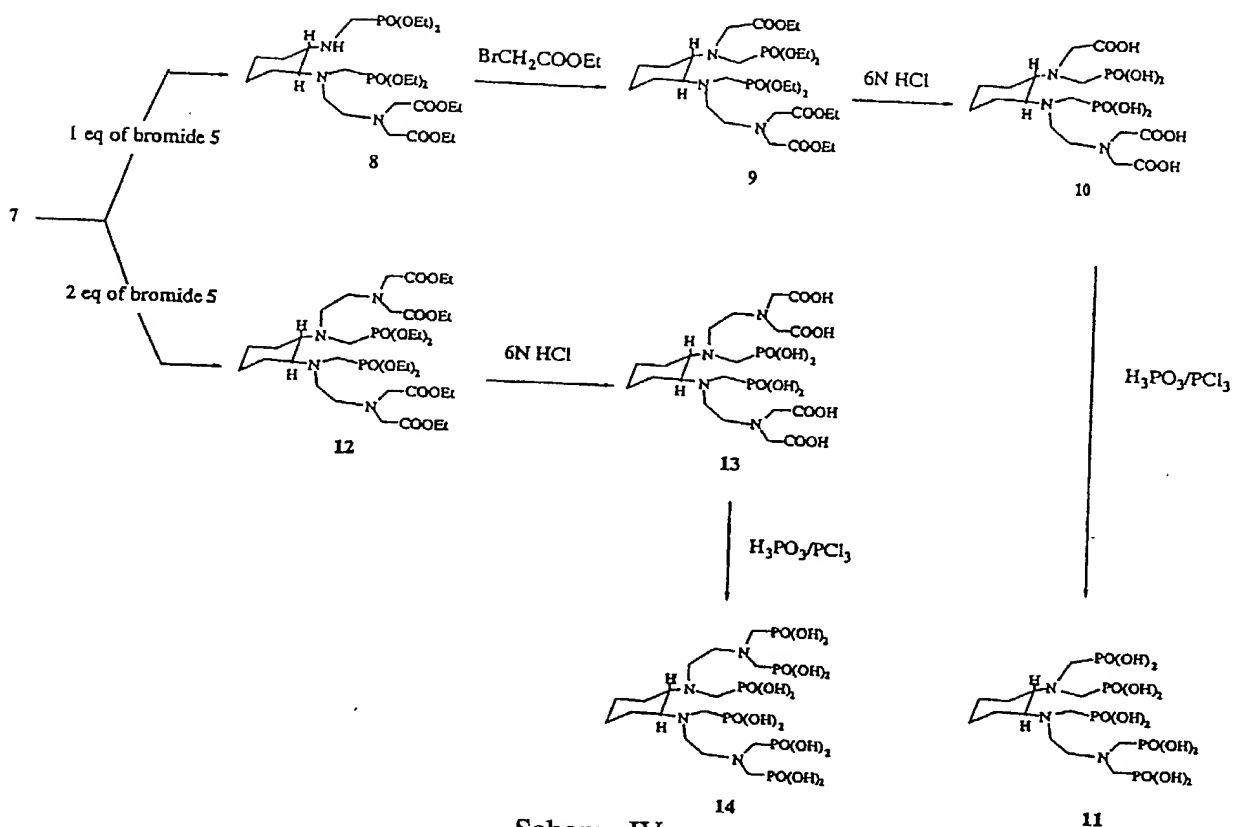
scheme II

- In the case of certain diamines as *trans*-1,2-diaminocyclohexane **1**, the second route, as shown in schemes III, IV allowed the aminophosphonomethylation of amines protected by a methylene bridge between the two nitrogen atoms of **1**. This protecting group will subsequently provide for a different functionalization on the amine. The reaction of Kabachnick-field described and detailed by Baily and Burgada (Baily and Burgada, 1995), gave compound **6** which was prepared from paraformaldehyde and diethylphosphite in THF. **7** was obtained by removing the protecting group in acidic conditions.



scheme III

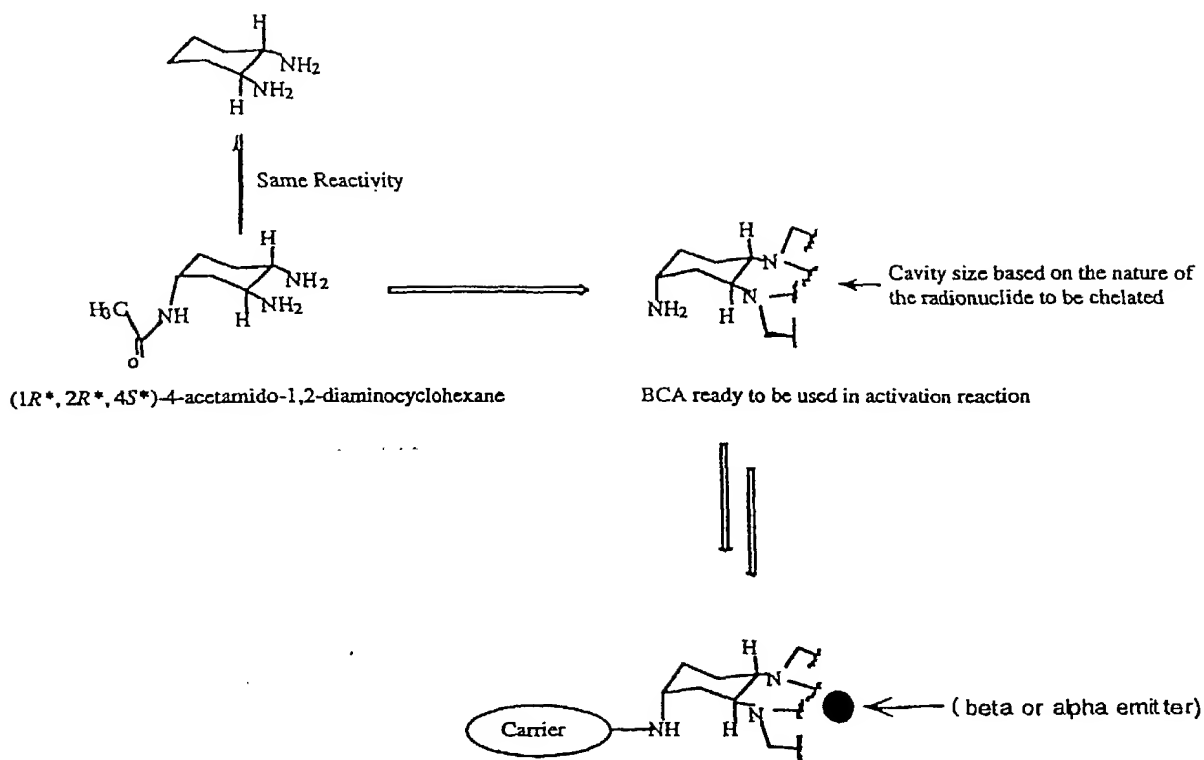
The monoalkylation or the dialkylation (see scheme IV), depending on the stoichiometry of the reaction gave respectively compounds **8** and **12**. The mixed acid **13** was obtained after hydrolysis of **12** in 6M hydrochloric acid and the hexaphosphonic acid **14** was prepared according to the method of Bauer and Kruger as described above. The synthesis of chelating agents **10** and **11** required an additional step which was the alkylation of **8** by ethyl bromoacetate to give the mixed ester **9**. Acid-catalysed hydrolysis gave **10** and **11** after reaction of conversion described all above.



Scheme IV

In conclusion, different non-functionalized ligands bearing aminophosphonate or aminocarboxylate chelate groups and mixed chelate groups were prepared and tested for their complexation properties with ^{153}Sm . The synthetic method described above was applied to the previously synthesized intermediate, the (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane, resulting in the synthesis of several polyaminocarboxylic acids, polyaminophosphonic acids and mixed semi-rigid functionalized ligands (BCA).

The different access routes to non-functionalized compounds described here were used without modifying the synthesis in order to obtain their functionalized homologues ready to be used in a coupling reaction as described in scheme V. We observed the influence of aminocarboxylic acid and aminophosphonic acid functions on the stability of the resulting complexes.



Scheme V

Experimental

General Procedures

All experiments were performed under nitrogen. Solvents were distilled prior to reactions. The primary chemicals used were commercial products (Sigma-Aldrich Company). Product purity and reaction progress were monitored on thin-layer chromatography (TLC) plates (60 F254, Merck), and liquid chromatography was carried out on a silica gel column (Merck 60,70-230 mesh). TLC revelation was performed under UV light (254 nm) or by iodine.

Nuclear Magnetic Resonance (NMR)

¹H and ¹³C NMR spectra were recorded on a Bruker AC 250 spectrometer (250 Mhz). Chemical shifts are reported in ppm to phosphoric acid as reference (85% H₃PO₄ in heavy water), positive values being downfield.

Chemical shifts (δ) are reported in ppm. Coupling constant J is reported in Hertz (Hz).

Mass Spectrometry (MS)

MS spectra were recorded on a Mat Finnigan LCQ Ion Trap mass apparatus using the electrospray method in negative or positive mode.

Starting Material

The bisphosphonate **6** was prepared in our laboratory according the synthesis procedure of Bailly and Burgada with minor modifications.

Synthesis and Spectroscopic data

N,N'-[(2-ethoxycarbonyl)eth-1-yl]-trans-cyclohexane-1,2-diamine 2a:

To freshly distilled *trans*-1,2-diaminocyclohexane **1** (1 ml, 8.33 mmol) in 50 ml of ethanol was added vinyl propionate (1.50 ml, 13.7 mmol) in one portion. After stirring 20h at room temperature, the reaction mixture was concentrated by rotary evaporation to yield a pale yellow oil (2.6 g, 8.32 mmol, 100%) which was used directly in the next step. ¹H NMR (CDCl₃): δ 1.22 (t, 12H), 1.67 (m, 2H), 1.82 (m, 2H), 2.06 (m, 2H+2H), 2.43 (t, 4H), 2.67 (dt, 2H), 2.98 (dt, 2H), 4.10 (q, 4H). ¹³C NMR (CDCl₃): δ 14.17, 24.31, 31.46, 35.34, 42.19, 60.23, 61.29, 172.69. (M+H⁺): 315

N,N'-[(2-diethylphosphono)eth-1-yl]-trans-cyclohexane-1,2-diamine 2b:

To freshly distilled *trans*-1,2-diaminocyclohexane **1** (1 ml, 8.33 mmol) in 50 ml of ethanol was added diethyl vinyl phosphonate (2.80 ml, 18.21 mmol). The reaction mixture was allowed to stir at reflux during 15 hours. After removal the solvent under reduced pressure, the resulting oil was purified by column chromatography (silica gel, CH₂Cl₂-EtOH 1:1) to give 2.9 g of a limpid oil (6.65 mmol, 80%). ¹H NMR (CDCl₃): δ 0.94 (m, 2H), 1.15 (m, 2H), 1.24 (t, 12H), 1.64 (m, 2H), 1.83-1.95 (m, 8H), 2.05 (m, 2H), 2.71 (dt, 2H), 2.97 (dt,

N,N'-(ethylacetate)-*N,N'*[(2-diethylphosphono)eth-1-yl]-*trans*-cyclohexane-1,2-diamine **3b**: The tetraester has been prepared as described above for compound from 1 g of compound 2b, Na₂CO₃ (0.70g, 6.60 mmol), KI (0.40, 2.40 mmol) and ethylbromoacetate (1.80 ml; 7.15 mmol). Purification by chromatography (SiO₂ (CH₂Cl₂-MeOH 99 : 1) gave 0.58 g of a pale yellow oil (0.94 mmol; 41%). ¹H NMR (CDCl₃): δ 1.11 (m, 4H), 1.25 (t, 6H), 1.29 (t, 12H), 1.70 (m, 2H), 1.85-2.10 (m, 4H+2H), 2.90 (t, 4H), 3.38 (d, 4H), 4.09 (m, 12H). ¹³C NMR (CDCl₃): δ 14.11, 16.31, 16.41, 25.63, 26.03 (JC-P : 135), 27.98, 44.86, 52.43, 60.26, 61.31, 61.41, 63.08, 172.46. (M+H)⁺: 615

General procedure for preparation of corresponding acids: *trans*-cyclohexane-1,2-diamine-*N,N'*-acetic-*N,N'*-propionic acid 4a and *trans*-cyclohexane-1,2-diamine-*N,N'*-acetic-*N,N'*-ethylphosphonic acid 4b:

Compound 3a or 3b (1 g) was dissolved in 6N aqueous hydrochloric acid (12ml) and heated to reflux overnight. The refrigerant was removed, and the reaction mixture was kept at 70°C to dryness. An additional aqueous hydrochloric acid 6N (12 ml) was then added, and the solution was heated to dryness. the residue was taken up in MeOH and evaporated under reduced pressure. This step repeated twice gave the corresponding acid as an off-white solid, which was dried under vacuum and kept under nitrogen.

Compound 4a: ¹H NMR (D₂O): δ 1.25-1.40 (m, 4H), 1.60-2.15 (m, 4H), 2.28 (m, 2H), 2.75 (t, 2H), 2.96 (t, 2H), 3.22 (m, 2H), 3.50-3.90 (m, 4H), 4.15 (s, 1H), 4.28 (s, 1H) ¹³C NMR (CDCl₃): δ 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.92, 175.03, 176.46 (M-H⁺): 373

Compound 4b: ¹H NMR (D₂O): δ 1.05-1.40 (m, 4H), 1.65-2.15 (m, 4H), 2.90-3.25 (m, 6H), 3.45-3.65(m, 2H), 3.70-3.95 (m, 2H). ¹³C NMR (CDCl₃): δ 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.33, 176.73 (M-H⁺): 445

***trans*-cyclohexane-1,2-diamine-*N,N'*-ethylphosphonic-*N,N'*-methylphosphonic acid 4c:** A mixture of compound 4c (0.5 g; 1.12 mmol) and phosphorous acid (0.202 g; 2.46 mmol) in 10 ml of dry toluene was heated to 80°C and stirred for 30 min. PCl₃ (0.22 ml; 2.46 mmol) was then added dropwise, and the reaction mixture was kept at this temperature for 20 hours before being cooled to room temperature. The solvent was discarded and the residual product dissolved in a small volume of water. After filtration, the filtrate was evaporated to give a residue which was purified by precipitation in warm acetone and collected by filtration. The purification step was repeated twice to give 4c which was dried under vacuum and kept under nitrogen (0.460 g; 0.83 mmol; 74%). ¹H NMR (D₂O): δ 1.15-1.65 (m, 4H), 1.75-2.10 (m, 2H), 2.15-2.40 (m, 6H), 3.00-3.60 (m, 10H). ¹³C NMR (CDCl₃): δ (M-H⁺): 517.

***N,N*-Bis(ethylacetate)-2-bromoethyl-amine 5:** Bromide 5 was synthesised in our laboratory according the synthesis procedure of Williams and Rapoport with minor modifications concerning the bis *N*-alkylated ethanolamine synthesis. To a 4°C solution of ethanolamine (6ml; 0.1 mol) in 100 ml of dried acetonitrile was added dropwise ethylbromoacetate (7.4 ml; 66 mmol) over a period of 20

min during which time a large quantity of precipitate formed. The mixture was allowed to stir for 2 hours at this temperature. The white solid was removed by filtration and washed with a small quantity of acetonitrile. The filtrate was concentrated under reduced pressure. The resulting liquid was taken up in CHCl_3 (100mL) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a liquid which was used directly in the next step (5.9 g; 25.32 mmol; 77%). The dialkylated ethanolamine and Ph_3P (7.72 g; 27.9 mmol) were dissolved in CH_2Cl_2 (100mL). The mixture was cooled in an ice bath and vigorously stirred while NBS (4.96 g; 27.9 mmol) was added in small portions. After the solution was stirred at 0°C for two hours, evaporation of the solvent gave a semisolid which was triturated with ether and the resulting solid was separated by filtration. the filtrate was evaporated to give an oil which was purified by column chromatography (silica gel, CH_3Cl). (6.14 g; 20.7 mmol; 62% overall) ^1H NMR (CDCl_3): d 1.26 (m, 6H), 3.15 (t, 2H, $J = 7.75$ Hz), 3.44 (t, 2H, $J = 7.75$ Hz), 3.59 (s, 4H), 4.15 (q, 4H). ^{13}C NMR (CDCl_3): d (M+H $^+$): 297

***N,N'*-(2-cyano)eth-1-yl]-*N,N'*-[*N''*,*N''*-bis-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 3d:** To a solution of compound 2d (1 g; 4.54 mmol) and bromide 5 (3 g; 10.13 mmol) in a mixed solvent system (CH₃CN-EtOH, 1:1) was added Na₂CO₃ (1.4 g; 13.20 mmol) and KI (0.75 g; 4.54 mmol). After stirring for 2 days at 70°C, the reaction mixture was filtered and concentrated under reduced pressure. The residue was taken up in CHCl₃ (200 ml) and washed with water. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure to give a yellow-brown oil. The crude product was purified by column chromatography (silica gel; CH₂Cl₂-EtOH 98:2). The fractions containing pure product were collected and dried to give an limpid oil (1.09 g; 1.68 mmol; 37%). ¹H NMR (CDCl₃): δ 1.13 (m, 4H), 1.27 (t, 12H), 1.73 (m, 2H), 1.86 (m, 2H), 2.30-2.85 (m, 4H+2H), 2.95 (m, 2H), 3.55 (s, 8H), 4.17 (m, 12H). ¹³C NMR (CDCl₃): δ 14.25, 18.63, 25.79, 27.24, 47.35, 48.98, 53.78, 55.43, 60.54, 62.64, 119.49, 171.11. (M+H⁺): 652

trans-cyclohexane-1,2-diamine-*N,N'*-propionic-*N,N'*-[*N''*,*N'''*-bis-(2-aminoethyl)]-tetra-acetic acid **4d**: this hexaacid has been prepared as described above for compounds **4a** & **4b** from 1 g of the ester **3d** and two volumes of 20 ml HCl (6N). ¹H NMR (D₂O): 1.20-2.00 (m, 10 H), 2.30 (m, 2H), 2.40-3.00 (m, 4H), 3.10-3.95 (m, 14 H), 4.20 (m, 4H) d ¹³C NMR (D₂O) : d 26.89, 30.49, 40.99, 52.00, 55.18, 55.47, 58.97, 165.50, 170.38. (M-H⁺): 547

N,N'-(diethylphosphono-methyl)-*N'*-(ethylacetate)-*N*-[*N''*,*N''*-bis-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine **9**: The tetraester is prepared as described above for compounds 3a & 3b from compound 8.

Na₂CO₃, KI and ethylbromoacetate (1 equivalent). Purification by chromatography (SiO₂) gave a oil.

N,N'-(diethylphosphono-methyl)-*N,N'*-[*N'',N''*-bis-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 12: The mixed ester is prepared as described above for compound 3d from bisphosphonate 7 (1g, 2.41 mmol), Na₂HPO₄ (1 g, 7.04 mmol) and 2 equivalents of bromide 5 1.80 g, 6.08 mmol). Purification by chromatography (SiO₂ (CH₂Cl₂-MeOH 95 : 5) gave a pale yellow oil (0.59 g, 0.70 mmol, 29%). ¹H NMR: 1.16 (m, 4H), 1.25 (t, 12H), 1.33 (t, 12H), 1.69 (m, 2H), 1.86 (m, 2H + 2H), 2.70-3.50 (m, 8H + 2H + 2H), 3.57 (s, 8H), 4.12 (m, 16H). (M+H⁺): 847

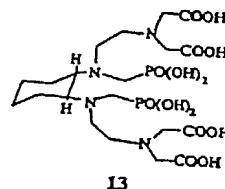
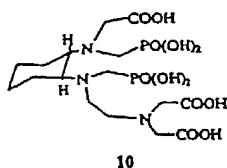
trans-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N-acetic*-,*N'*-[*N'',N''*-bis-(2-aminoethyl)]-tetra-acetic acid 10 & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-bis-(2-aminoethyl)]-tetra-acetic acid 13 : Those compounds are prepared as described above for compound 4a, 4b and 4d.

Compound 13: ¹H NMR (D₂O): 1.26 (m, 2 H), 1.45 (m, 2H), 1.75-2.00 (m, 4H), 2.80 (m, 2H), 3.00-3.70 (m, 12H), 4.10 (br s, 8H). (M-H⁺): 619

trans-cyclohexane-1,2-diamine-*N,N,N'*-methylphosphonic-*N'*-[*N'',N''*-bis-(2-aminoethyl)]-di-methylphosphonic acid 11 & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-bis-(2-aminoethyl)]-tetra-methylphosphonic acid 14 : Those compounds are prepared as described above for compound 4c.

153Sm complexation studies

Complexation studies with Samarium 153 were performed on the two following chelating agents 10 (AL 247) and 13 (AL 245)



Radiochemistry purity was measured on ITLC-SG chromatographic profiles. Radioactivity was quantified using a Phosphorimager 445SI apparatus.

Samarium 153 was furnished under $^{153}\text{SmCl}_3$ form in HCl 0,04N with a 5,2 GBq/ml volumic activity and a 40 GBq/mg specific activity.

They were tested for their complexation properties by using an excess of 10 to 50 equivalents of chelating agent.

Competition studies were performed against EDTMP according to the following method :

- first step : 50 equivalents of one of the chelating agents and a fixed amount of ^{153}Sm were incubated at 37°C during 3 hours in order to form the ^{153}Sm -CA complex (CA = Chelating Agent),

- second step : 50 equivalents of EDTMP were added to the previous solution and kept 3h at 37°C in order to measure the decomplexation. Another measure was performed 72h after to ensure a complete decomplexation possibility.

Results :

% of non decomplexed $^{153}\text{Sm-CA}$

chelating agent

after 3h

after 72h

10 (AL 247)

100

67

13 (AL 245)

100

100

AL 247 and AL 245 present very good complexation properties for ^{153}Sm and in any cases better than EDTMP

Furthermore, stability was performed on AL 245 in human serum media at 37°C at different time and showed no loss of 153Sm from AL 245 at either 24, 48, 72 and 96h.

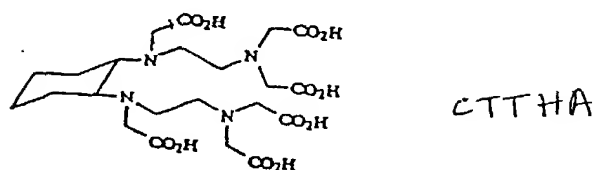
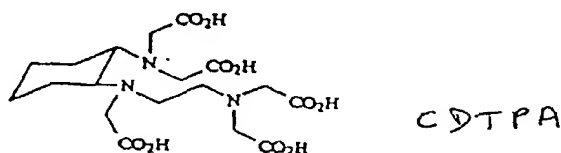
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. a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

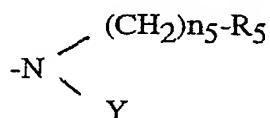
a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



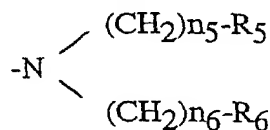
2. Compounds according to claim 1, characterized in that :

- 20
- when R₁, R₂, R₃ or R₄ represents -COOH or -PO(OH)₂, then n₁, n₂, n₃ or n₄ represents 1 respectively,
 - when R₁, R₂, R₃ or R₄ represents a group



- 25
- then n₁, n₂, n₃ or n₄ represents 2 or 3 respectively, and preferably 2,
 - n₅, and optionally n₆, represents 1.

3. Compounds according to claims 1 or 2, characterized in that at least one, and more preferably two of R₁, R₂, R₃ and R₄, represent a group



wherein n₅, n₆, R₅ and R₆ are defined in claims 1 or 2.

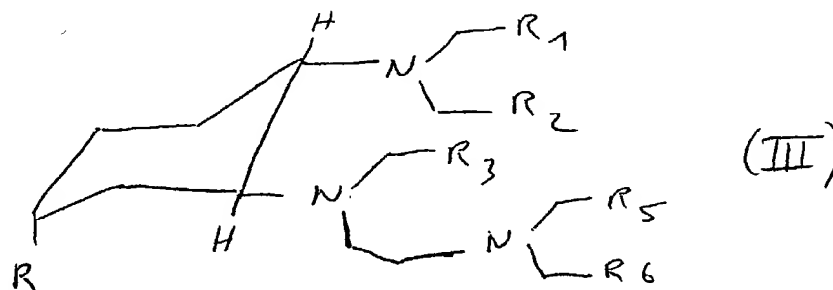
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4. Compounds according to anyone of claims 1 to 3, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in

claim 1, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding.

5 5. Compounds according to anyone of claims 1 to 3, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in claim 1.

10 6. Compounds according to anyone of claims 1 to 5 of the following formula (III) :

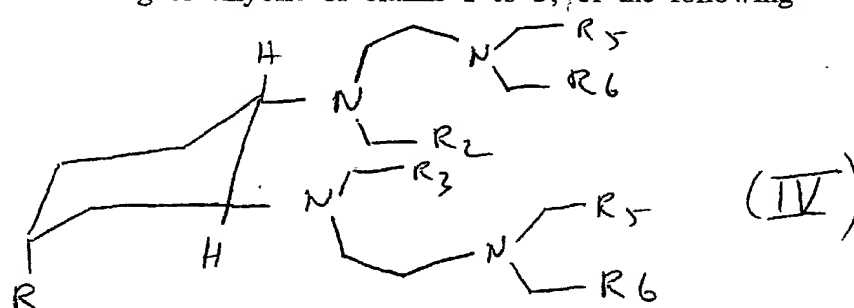


20 in which R₁, R₂, R₃, R₅ and R₆ independently from each other represent -COOH or -PO(OH)₂, and R is a group as defined in claims 2 to 5.

25 7. Compounds according to claim 6, of formula (III) wherein :

- . R₁ = R₅ = R₆ = COOH and R₂, = R₃ = PO(HO)₂, or
- . R₁ = R₂ = R₃ = R₅ = R₆ = COOH, or
- . R₁ = R₂ = R₃ = R₅ = R₆ = PO(OH)₂.

30 8. Compounds according to anyone of claims 1 to 5, of the following formula (IV) :



35 wherein R₂, R₅ and R₆, independently from each other, represent -COOH or -PO(OH)₂, and R is a group as defined in claims 2 to 5.

9. Compounds according to claim 8 of formula (IV) wherein :

- . $R_2 = R_3 = PO(OH)_2$, and $R_5 = R_6 = COOH$, or
- . $R_2 = R_3 = R_5 = R_6 = COOH$, or
- . $R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

10. Complexes between a compound according to anyone of claims 1 to 9, and a radioactive element.

11. Complexes according to claim 10, characterized in that said radioelements are α or β emitter radiometals.

12. Complexes according to claim 11, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,
- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound, in a first step of the treatment, to epitopes on the surface of specific cells in the organism,
- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

13. Use of a complex according to claims 11 or 12, for the manufacture of a medicament for radioimmunotherapy, such as for the treatment of cancers, and more particularly for the treatment against metastase proliferation.

14. Pharmaceutical compositions characterized in that they comprise an effective amount of a complex according to claims 11 or 12, in association with a suitable pharmaceutical carrier.

15. Complexes according to claim 10, characterized in that the radioelements are γ emitter radiometals.

16. Complexes according to claim 15, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

17. Use of a complex according to claims 15 or 16, for carrying out diagnosis methods such as radioimmunoscintigraphy.

18. Use of a compound of formula (I) as defined in claim 1 to 9, included compounds CDTPA and CTTHA, for :

- the manufacture of a medicament useful as antalgic, or for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism,

- carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not,

- carrying out a process for the radionuclides purification, said compound being bound to a solid phase,

- carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

ABSTRACT OF THE DISCLOSURE

The invention relates to compounds of formula (I):
in which : n_1 , n_2 , n_3 and n_4 , represent an integer from 1 to 5,
 R_1 , R_2 , R_3 , and R_4 , independently from each other, represent
-COOH, -PO(OH)₂ at least R_1 , R_2 , R_3 , or R_4 represents a group
5 (II), wherein n_5 represents an integer from 1 to 5, R_5
represents -COOH or -PO(OH)₂, and Y represents H or a group
-(CH₂) n_6 - R_6 in which n_6 represents an integer from 1 to 5, and
 R_6 represents -COOH or -PO(OH)₂, R represents H, or a group
carrying a function linked to molecules able to bind with
10 epitopes at the surface of cells. The invention also relates
to the processes of preparation of the compounds, and to their
use in pharmaceutical compositions and in diagnosis methods.

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

the specification of which: *(check one)*

REGULAR OR DESIGN APPLICATION

- ☐ is attached hereto.
- ☐ was filed on _____ as application Serial No. _____ and was amended on (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

- ☒ was described and claimed in International application PCT/EP99/08031 filed on 22 October 1999 and as amended on (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

PRIORITY CLAIM

I hereby claim foreign priority benefits under 35 USC 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)

Country	Application Number	Date of Filing (day, month, year)	Priority Claimed
Europe	98402648.4	23 October 1998	yes

(Complete this part only if this is a continuing application.)

I hereby claim the benefit under 35 USC 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)

(Filing Date)

(Status--patented, pending, abandoned)

POWER OF ATTORNEY

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from Grosset-Fournier & Demachy as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

As a named inventor, I hereby appoint the registered patent attorneys represented by Customer No. **000466** to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, including: **Robert J. PATCH**, Reg. No. 17,355, **Andrew J. PATCH**, Reg. No. 32,925, **Robert F. HARGEST**, Reg. No. 25,590, **Benoît CASTEL**, Reg. No. 35,041, **Eric JENSEN**, Reg. No. 37,855, **Thomas W. PERKINS**, Reg. No. 33,027, and **Roland E. LONG, Jr.**, Reg. No. 41,949,

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

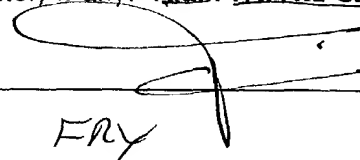
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U.S. APPLICATION NO. (if known, see 37 CFR 1.41) <div style="font-size: 2em; font-weight: bold; margin-left: 100px;">097830188</div>		INTERNATIONAL APPLICATION NO. PCT/EP99/08031		ATTORNEY'S DOCKET NO. 98 BA INS SAM	
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17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$ 1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$ 860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$ 710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$ 690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$ 100.00 <div style="text-align: right; margin-right: 50px;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>	CALCULATIONS PTO USE ONLY																																																																			
	\$	860.00																																																																		
Surcharge of \$130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	\$	130.00																																																																		
<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <th style="width:15%;">CLAIMS</th> <th style="width:15%;">NUMBER FILED</th> <th style="width:15%;">NUMBER EXTRA</th> <th style="width:15%;">RATE</th> <th style="width:10%;">\$</th> <th style="width:30%;"></th> </tr> <tr> <td>Total claims</td> <td>18 - 20 =</td> <td>0</td> <td>X \$18.00</td> <td>\$</td> <td></td> </tr> <tr> <td>Independent claims</td> <td>1 - 3 =</td> <td>0</td> <td>X \$80.00</td> <td>\$</td> <td></td> </tr> <tr> <td colspan="3">MULTIPLE DEPENDENT CLAIMS(S) (if applicable)</td> <td>+ \$270.00</td> <td>\$</td> <td></td> </tr> <tr> <td colspan="4" style="text-align: right;">TOTAL OF ABOVE CALCULATIONS =</td> <td>\$</td> <td style="text-align: right;">990.00</td> </tr> <tr> <td colspan="4">Reduction of 1/2 for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27.</td> <td style="text-align: center;">+</td> <td></td> </tr> <tr> <td colspan="4" style="text-align: right;">SUBTOTAL =</td> <td>\$</td> <td style="text-align: right;">990.00</td> </tr> <tr> <td colspan="4">Processing fee of \$130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.49(f)).</td> <td>\$</td> <td></td> </tr> <tr> <td colspan="4" style="text-align: right;">TOTAL NATIONAL FEE =</td> <td>\$</td> <td style="text-align: right;">990.00</td> </tr> <tr> <td colspan="4">Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property</td> <td style="text-align: center;">+</td> <td></td> </tr> <tr> <td colspan="4" style="text-align: right;">TOTAL FEES ENCLOSED =</td> <td>\$</td> <td style="text-align: right;">900.00</td> </tr> </table>	CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$		Total claims	18 - 20 =	0	X \$18.00	\$		Independent claims	1 - 3 =	0	X \$80.00	\$		MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ \$270.00	\$		TOTAL OF ABOVE CALCULATIONS =				\$	990.00	Reduction of 1/2 for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27.				+		SUBTOTAL =				\$	990.00	Processing fee of \$130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.49(f)).				\$		TOTAL NATIONAL FEE =				\$	990.00	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				+		TOTAL FEES ENCLOSED =				\$	900.00	Amount to be refunded: charged:	
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a.	<input checked="" type="checkbox"/>	A check in the amount of \$ 990.00 to cover the above fees is enclosed.
b.	<input type="checkbox"/>	Please charge my Deposit Account No. 25-0120 in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed.
c.	<input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. 25-0120 . A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

Customer No. 000466

YOUNG & THOMPSON

745 South 23rd Street

2nd Floor

Arlington, VA 22202

(703) 521-2297 facsimile (703) 685-0573

By *Benoît Castel*

Benoît Castel

Attorney for Applicants

Registration No. 35,041

April 23, 2001